J. Parasitol., 90(5), 2004, pp. 1015–1018

TISSUE DISTRIBUTION AND MOLECULAR CHARACTERIZATION OF CHICKEN ISOLATES OF *TOXOPLASMA GONDII* FROM PERU

J. P. Dubey, M. Z. Levy*†, C. Sreekumar, O. C. H. Kwok, S. K. Shen, E. Dahl*, P. Thulliez‡, and T. Lehmann*

United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. *e-mail: jdubey@anri.barc.usda.gov*

ABSTRACT: The prevalence of *Toxoplasma gondii* in free-ranging chickens is a good indicator of the prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The prevalence of *T. gondii* antibodies in sera of 50 free-range chickens (*Gallus domesticus*) from Peru was 26% on the basis of the modified agglutination test (MAT). Hearts, pectoral muscles, and brains of seropositive (MAT ≥1:5) chickens were bioassayed individually in mice. Tissues from the remaining 37 seronegative chickens were pooled and fed to 2 *T. gondii*–free cats. Feces of cats were examined for oocysts; they did not shed oocysts. *Toxoplasma gondii* was isolated from the hearts of 10 seropositive chickens but not from their brains and pectoral muscles. Genotyping of these isolates using the SAG2 locus indicated that 7 isolates were type I and 3 were type III. Six of the 7 type-I isolates were avirulent for mice, which was unusual because type-I isolates are considered virulent for mice. The *T. gondii* isolates were from chickens from different properties that were at least 200 m apart. Thus, each isolate is likely to be different. This is the first report of isolation of *T. gondii* from chickens from Peru.

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts in the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or to other factors.

Toxoplasma gondii isolates have been classified into 3 genetic types (I, II, III) on the basis of restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997). It has been suggested that type-I strains or recombinants of types I and III are more likely to result in clinical ocular toxoplasmosis (Howe et al., 1997; Fuentes et al., 2001; Grigg et al., 2001; Aspinall et al., 2003), but genetic characterization has been limited essentially to isolates from patients ill with toxoplasmosis. Unlike these reports, Ajzenberg et al. (2002) found that most (73 of 86) isolates from cases of congenital toxoplasmosis in humans from France were type II. Nothing is known of the genetic diversity of *T. gondii* isolates circulating in the general human population. In animals, most isolates of *T. gondii* were type II or III, irrespective of clinical status (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002). *Toxoplasma gondii* isolates differ markedly in their virulence to outbred mice. Type-I isolates are more virulent to mice than types II and III. Because chickens become infected mostly by feeding from ground contaminated with oocysts, prevalence of *T. gondii* in chickens is a good indicator of the strains prevalent in their environment (Ruiz and Frenkel, 1980).

Recently, we found that 70% of 73 *T. gondii* isolates obtained from asymptomatic free-range chickens from Brazil were type I (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003), whereas samples from Egypt and the United States were predominantly either type II or III but not type I (Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, Dahl, Sreekumar et al., 2003). The type-II isolates of *T. gondii* have not been found in chickens from Brazil. All 3 types were found in chickens from Argentina (Dubey, Venturini et al., 2003). Nothing is known of the characteristics of isolates of *T. gondii* from animals or humans from Peru. In the present article, we attempted to isolate and genotype *T. gondii* from chickens from Peru. In addition, the distribution of *T. gondii* in the heart, brain, and pectoral muscles of chickens was compared.

MATERIALS AND METHODS

Chickens from Peru

The chickens (n = 50) were from a periurban shantytown in the desert hills around San Juan de Miraflores, Lima, Peru. Each chicken was from a different property located at the height of 103–300 m above sea level and between longitudes and latitudes of 76°57′135″–76°58′048″W and 12°10′435″–12°11′429″S. The properties were approximately 200 m apart. Chickens were purchased, and killed by cervical dislocation from 26 to 28 September 2003. Blood was collected, centrifuged, and samples of serum, heart, pectoral muscle, and brain from each chicken were sent cold, by air to Beltsville, Maryland. Four to 6 days elapsed between killing of chickens and receipt of samples at Beltsville.

Serological examination

Sera of chickens were tested for *T. gondii* antibodies using 4 serum dilutions, 1:5, 1:10, 1:20, and 1:200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). After the completion of the bioassays, all seropositive chicken sera were rerun using 2-fold dilutions from 1:5 to 1:320.

Bioassay of chickens for infection

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains, pectoral muscles, and hearts of seropositive (MAT 1:5 or more) chickens were each bioassayed individually in outbred female Swiss Webster mice obtained from Taconic

Received 30 January 2004; revised 23 February 2004; accepted 25 February 2004.

* Division of Parasitic Diseases, Centers for Disease Control and Prevention, Chamblee Campus, Mail Stop F22, 4770 Buford Highway, Atlanta, Georgia 30341.

† Program in Population Biology, Ecology, and Evolution, Emory University, Atlanta, Georgia 30322.

‡ Laboratoire de la Toxoplasmose, Institut de Puericulture, 26 Boulevard Brune, Paris-75014,, France.

TABLE I. Isolation of *Toxoplasma gondii* from hearts of chickens from Peru.

		T. gondii isolation in mice			
Chicken no.	Antibody titer	No. of mice *T. gondii* positive*	No. of mice that died	Day of death	Genotype
2	≥320	5	5	17, 18, 18, 18, 19	I (TgCkPe1)
7	20	5	0	NA†	III (TgCkPe2)
10	≥320	5	1	30	III (TgCkPe3)
14	≥320	5	0	NA	I (TgCkPe4)
23	160	5	0	NA	I (TgCkPe5)
27	≥320	5	2	25, 26	I (TgCkPe6)
36	160	4	0	NA	I (TgCkPe7)
39	≥320	2	1	23	I (TgCkPe8)
40	≥320	5	1	47	I (TgCkPe9)
46	≥320	3	0	NA	III (TgCkPe10)

* Of 5 mice inoculated.
† Not applicable.

Farms, Germantown, New York, as described (Dubey et al., 2002). Each tissue was homogenized individually, digested in acidic pepsin, washed, and homogenate inoculated subcutaneously into 5 mice; in total, 15 mice were inoculated with tissues of each chicken. Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 39 postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 53 days PI, and their brains were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

Tissues from 37 seronegative chickens were pooled in 2 batches of 20 and 17 chickens and fed separately to 2 *T. gondii*–free cats (Dubey et al., 2002). Brain, lungs, and heart of 4 of the 5 mice that died 17–19 days PI with tissues of chicken no. 2 (Table I) were fed to a cat. Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days after ingestion of chicken tissues as described previously (Dubey, 1995). The total number of oocysts shed by each cat was counted as described (Dubey et al., 2002). Oocysts were allowed to sporulate for 1 wk at room temperature, and sporulated oocysts were bioassayed in mice (Dubey and Beattie, 1988).

Genetic characterization for *T. gondii*

A portion of lungs or brain was removed from mice that died within 4 wk PI of chicken tissues and was frozen at −70 C for DNA characterization. Mesenteric lymph nodes of mice that died or were killed 4 days after being fed oocysts were removed for *T. gondii* DNA isolation. *Toxoplasma gondii* DNA was extracted from mouse tissue as described previously (Lehmann et al., 2000). The RFLP strain type of *T. gondii* isolates was determined by nested polymerase chain reaction on the SAG2 locus according to Howe et al. (1997).

RESULTS

Antibodies to *T. gondii* were found in 14 of 50 chickens with titers of 1:5 in 1, 1:20 in 3, 1:160 and in 2, and 1:320 or more in 7. *Toxoplasma gondii* was isolated only from the hearts of 10 of 13 seropositive chickens; from 1 of 3 with titer of 1:20, from 2 of 3 with titer of 1:160, and from all 7 with titers of 1:320 or more (Table I). The isolate of *T. gondii* from chicken no. 2 killed all 5 infected mice within 19 days PI (Table I). Mice inoculated with tissues from chickens nos. 10, 14, 23, 27, 36, and 40 were lethargic during the second and third week PI, but only a total of 5 (15%) of them died (Table I). The 2 cats fed tissues of 37 seronegative chickens did not shed oocysts. The cat fed tissues of acutely infected mice inoculated with tissues of chicken no. 2 shed more than 10 million oocysts. Genotyping of *T. gondii* isolates from chickens indicated that 7 isolates were type I and 3 were type III (Table I).

DISCUSSION

The threshold MAT titer indicative of *T. gondii* infection in chickens has not been determined. Data comparing serology and recovery of viable *T. gondii* from chickens are now accumulating (Dubey et al., 2002; Dubey, Graham, Dahl, Hilali et al., 2003; Dubey, Graham, da Silva et al., 2003; Silva et al., 2003). Although *T. gondii* has been isolated from a few chickens with MAT titers of 1:5 or less, the likelihood of isolation increased with higher MAT titer. The MAT test used in the current study is at present the best assay for detecting antibodies to *T. gondii* in chickens (Dubey et al., 1993). Unlike in other hosts, the classic Sabin–Feldman dye test does not detect antibodies to *T. gondii* in chickens (Dubey et al., 1993). Ruiz and Frenkel (1980) isolated *T. gondii* from 27 of 54 chickens from Costa Rica that had no detectable dye test antibodies in 1:2 dilution of serum. Lack of shedding of oocysts by cats that consumed entire hearts, brains, and 20–25 g of pectoral muscles of 37 seronegative chickens supports the validity of the MAT.

Notably, *T. gondii* was isolated from the hearts but not from the brain and pectoral muscles of the chickens. *Toxoplasma gondii* is regarded as a neurotropic organism. However, this assumption is based on the studies in mice and because humans can develop severe neural toxoplasmosis. Studies in other asymptomatic animals have questioned this convention (Jacobs et al., 1963; Dubey and Beattie, 1988; Dubey, 1997). Jacobs and Melton (1966) isolated *T. gondii* from 4 of 108 chickens from a slaughterhouse in Maryland and from ovaries of 3 and leg muscles of 1, but not from the brain of any chicken. Dubey

(1981) isolated *T. gondii* from 3 of 11 free-range chickens from a farm in Montana and from hearts of all 3 and brain of 1, but not from their lungs, spleen, liver, kidneys, or pectoral muscles. In the present study, tissues from chickens were reasonably fresh and thus provided an opportunity to compare tissue tropism. It is clear from these studies that hearts should always be included among tissues in attempts to isolate *T. gondii* from chickens. The amount of material bioassayed did not affect the results because the entire brains and hearts were bioassayed; approximate weights of each brain, heart, and pectoral muscles bioassayed were 3, 5, and 10 g, respectively.

The mouse virulence and genotyping data indicate that the isolates of *T. gondii* from Peru are different from isolates from other countries, even in the Americas. The lineage composition in Peru was similar to that in Brazil (Dubey et al., 2002; Dubey, Graham, da Silva et al., 2003; Dubey, Navarro et al., 2003) and consisted of 70% type I and 30% type III (Table I). However, most Brazilian isolates were virulent for mice, irrespective of the genotype, whereas most Peruvian isolates were avirulent for mice (Table I). In contrast, all 3 types were isolated from Argentina (Dubey, Venturini et al., 2003). None of the isolates from Egypt (Dubey, Graham, Dahl et al., 2003), India (Sreekumar et al., 2003), Mexico (Dubey et al., 2004), or the United States (Dubey, Graham, Dahl, Sreekumar et al., 2003; Lehmann et al., 2003) was virulent for mice. Only 1 of 10 isolates from Peru was mouse virulent, indicating that there are differences among isolates obtained from the 3 South American countries. The type-I strain has not been found in chickens in the United States or Egypt.

The cat fed tissues of mice that died 17–19 days PI with chicken heart shed millions of oocysts, confirming our earlier findings that oocyst shedding is independent of the mouse virulence of the strain and that asymptomatic chickens can harbor mouse virulent strains of *T. gondii* (Dubey et al., 2002). Until now, all type-I strains of *T. gondii* were considered to be lethal to mice, irrespective of dose of inoculation. The results of the present study and those reported earlier (Dubey, Graham, da Silva et al., 2003; Dubey et al., 2004) indicate that on the basis of SAG2 typing, not all type-I strains are mouse virulent.

ACKNOWLEDGMENTS

The authors thank Lilia Cabrera, A. Gilborn, and K. Hopkins for technical assistance. M.Z.L. was supported by a Howard Hughes Predoctoral Fellowship.

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